Osteoporosis and transforming growth factor-beta-1 gene polymorphism in Chinese men and women

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Abstract Transforming growth factor-beta-1 (TGF-β1) has been implicated in bone mineral density (BMD) determination. We investigated the relationship between the TGF polymorphism, BMD, and vertebral fractures in 588 Chinese men and women. No association between TGF polymorphism and BMD was observed in postmenopausal women (aged 55–59 years), elderly men (aged 70–79 years), or elderly women (aged 70–79 years) at the hip, spine, or total body (P > 0.05 by two-way ANOVA). In all study groups, there was no effect of an interaction between TGF polymorphism and calcium intake on BMD (P > 0.05 for the interaction effects by two-way ANOVA). No statistical significant association was observed between TGF polymorphism and vertebral fracture in elderly men or women (P > 0.05 by the chi-square test), even though men of the TT and TC genotypes seem to have more vertebral fractures. Contrary to previous studies that found an association between BMD and TGF polymorphism in the Japanese, we found no association between TGF polymorphism and BMD of elderly Chinese men or women. This finding could result from different sampling methods between the previous and current studies and environmental factors and ethnic differences between the two populations.

Key words transforming growth factor-beta-1 (TGF-β1) · osteoporosis · Bone mass · Chinese · polymorphism · gene

Introduction Osteoporosis is a multifactorial disease affected by both genetic and environmental factors [1,2]. Results from twin and family studies [3] have shown that genetic factors account for 50%–85% of the phenotypic variance in osteoporosis. However, polymorphic markers commonly used in other populations in osteoporosis genetic research may not be useful genetic makers in the Chinese population, as many of these markers are either absent or too rare in Chinese [4]. We have previously studied the relationship between the vitamin D receptor gene and estrogen receptor gene with BMD in Chinese, with mainly negative results [5–7].

Transforming growth factor-beta-1 (TGF-β1) is abundant in bone and plays an important part in regulating bone resorption and formation. The role of TGF-β1 in determining bone mineral density and frequency of fractures was recently studied in different populations [8–12]. Yamada et al. [10,11] showed an association between T → C transition in exon 1 of the TGF-β1 gene and bone mineral density in both postmenopausal Japanese women and adolescents. Moreover, the same polymorphism was found to be associated with frequency of vertebral fractures, with higher frequency of fractures found in individuals with the T allele than in those without [12].

We describe here the first study on the relationship between TGF polymorphism and BMD in Chinese. The relationship between the TGF-β1 polymorphism and vertebral fracture was also studied.

Materials and methods

Study subjects

Three groups of Chinese subjects were studied, women aged 55–59 years (n = 149), women aged 70–79 years (n = 207), and men aged 70–79 years (n = 232). The study subjects were community dwelling and were recruited through social centers in the district of Shatin. The older men and women were not selected with respect to fracture status, but X-rays were taken to determine their status for vertebral fracture. The detailed methods are discussed in a following section.
Subjects with a known history of metabolic bone disease and hip fracture were excluded. Subjects who had received drug treatment in the form of bisphosphonates, calcitonin, fluoride, or hormone replacement therapy were also excluded. The study was approved by the Chinese University of Hong Kong Clinical Research Ethics Committee, and all subjects gave informed consent.

**Determinant of TGF-β genotype frequencies in Chinese**

Genomic DNA was extracted from 10 ml of peripheral leukocytes by a standard phenol:chloroform procedure according to the method established by Yaich et al. [13]. We amplified exon 1 of the TGF-β gene by polymerase chain reaction (PCR) with genomic DNA (0.1 µg) in 20 µl buffer solution (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 2.0 mM MgCl2, 200 µM each of the four deoxyribonucleotides). One unit of Taq polymerase (Roche Molecular Biochemicals, Indianapolis, IN, USA), 1.4% dimethylsulfoxide (DMSO), 0.01% gelatin, 1.0 M betanine, and 0.4 µM each of oligonucleotide primer (sense primer 5′-TCCTACCTTTTGCCGGAGAC-3′) and antisense primer (antisense primer 5′-GTTGTGGGTITCACCATTAG-3′) was performed for one cycle with an initial step at 95°C for 4 min, 58°C for 1 min, and 72°C for 1 min; 30 cycles of 94°C for 45 s, 53°C for 30 s, and 72°C for 45 s; and a final extension at 72°C for 10 min. The PCR products were visualized by 1.5% agarose gel electrophoresis with ethidium bromide. The expected size of the specific amplification product was 346 bp. The PCR products were purified with Microspin Amersham Pharmacia S-300 HR columns (Amersham Pharmacia, Princeton, NJ, USA) and directly sequenced on the sense strand with a fluorescence-based automated DNA sequencer ABI-310 (Applied Biosystems, Foster City, CA, USA).

**Dual-energy X-ray absorptiometry measurements**

Bone mineral density (BMD) of the posteroanterior lumbar spine (L1–L4) and total hip (femoral neck, trochanter, and intertrochanter) were measured by dual-energy X-ray absorptiometry (QDR-2000; Hologic, Bedford, MA, USA). The coefficients of variation for BMD measurements in our laboratory were 0.8% for the total body, 0.7% for the spine, 1.2% for the femoral neck, and 1.4% for the intertrochanteric area.

**Statistical analysis**

The relationship between the TGF-β1 genotypes and BMD was studied by analysis of variance (ANOVA). As there was no significant difference in height, weight, and calcium intake between the TGF-β1 genotypes, no adjustments were made when BMD was studied. The significance of interaction between calcium intake and TGF genotype was also tested for in two-way ANOVA.

**Diagnosis of vertebral fracture**

The methods for the diagnosis of vertebral fracture have been described in detail previously [14]. In brief, lateral thoracic and lumbar spine films were taken (T4–L4). The films were digitized, and subjects with anterior to posterior, middle to posterior, or posterior to posterior (to the vertebra above and below) vertebral height ratios of 3 standard deviations or more below the population mean were considered to have vertebral fractures.

**Results**

The body weight, height, calcium intake, age, and BMD in the three TGF-β1 genotypes are shown in Table 1. The frequency distribution of the TGF-β1 genotypes in different sex and age groups was in Hardy–Weinberg equilibrium. The prevalence of the TT genotype in postmenopausal women, elderly Chinese women, and elderly Chinese men was 16%, 17%, and 17%, respectively. There was no significant association between BMD at all sites and TGF-β1 genotypes in postmenopausal women, elderly Chinese women, or elderly Chinese men (P > 0.05). Owing to the small number of subjects with the TT genotype, and previous studies showing that subjects carrying a T genotype had lower bone density, the subjects of TT and TC genotype were pooled (Table 2). In elderly men, significant difference between subjects with and without the T allele was found at the intertrochanteric site (P < 0.05), with higher BMD found in men with the T allele.

The distribution of the TGF-β1 genotype in elderly women and men with and without vertebral fracture is shown in Table 3. Although men of the TT and TC genotypes seem to have more vertebral fractures, this was not statistically significant. Moreover, there was no significant difference between vertebral fracture patients and the controls of either sex in their body height, body weight, age, and calcium intake levels.

**Discussion**

Transforming growth factor is produced by osteoblasts and has been implicated in the regulation of bone metabolism. It is released from bone matrix during bone resorption when activated by the acidic microenvironment created by osteoclasts and is also important in the proliferation and differentiation of osteoblasts [9].